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   The Influence of Some Vitamins on the Physiology of Colorado Potato Beetle /Leptinotarsa decemlineata Say/

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II. S U M M A R Y

Tocopherols - vitamin E, and unsaturated fatty acids - vitamin F are the compounds of great physiological importance. These compounds are necessary for proper operating in some metabolic processes especially for lipid metabolism. The aim of our researches is to explain what part these compounds are playing in Colorado beetle development. The biological experiments on the fertility, mortality and other characteristics of Colorado beetle activities as well as biochemical analyses of lipid metabolism will disclose us the proceeding way of these vitamins. To find the influence of these constituents on Colorado beetle development we carried out the biological experiments in insectarium on larvae and then on the adult beetles in the five different breeding combinations:

1/ We breed the larvae and the beetles with potato leaves enriched by adding alfa tocopherol,

2/ by adding unsaturated fatty acids,

3/ by adding the mixture of alfa tocopherol and unsaturated fatty acids,

4/ with the leaves sprayed only with the solvent - ether, and

5/ the control.

During the whole cycle of development of Colorado beetle we recorded data of the following observations: fertility, mortality, eating habits and other characteristics of their behaviour. On the larvae L, and on the beetles from five breeding combinations the following analyses were made: the determination of the total lipids, the spectrophotometric determination of unsaturated fatty acids - linoleic, linolenic, arachidonic and pentaenoic. The qualit. chromatographic determination of fatty acids was made too. We have made also the analyses of total tocopherols in the hemolymph and in the whole body of the larvae L, /breeded only with leaves of potato Dar manioty/. The results of our experiments have proved that while enriching the food-stuff for Colorado beetle with vitamins mentioned above, the number of eggs laid by the female beetles increased considerably. Also the biochemical analyses have shown the differences in content of unsaturated fatty acids in the larvae and beetles lirids. The content of the unsaturated fatty acids was found higher where vitamins treatment was applied, and also it was higher in the larvae lipids than in the beetles. That means that the unsaturated fatty acids are used by larvae during the chrysalis period. As it was stated in the quality chromatographic analyses the composition of fatty acids in the larvae and in beetles in all breeding treatments remains the same. There are some quantities of saturated fatty acids /palmitic and stearic/ but chiefly the unsaturated fatty acids /oleic, linoleic, linolenic and others/. We came to the following conclusion that the tocopherols /vitamin E/

and unsaturated fatty acids /vitamin F/ in metabolic processes of Colorado beetles play extremely important role.

III. D e t a i l e d r e p o r t

1. I n t r o d u c t i o n

Many foreign researchers have expressed in the literature their opinions about physiological importance of unsaturated fatty acids where they underline the great role of these acids in lipid metabolism questions /1, 2, 5, 7, 13, 15, 23, 22/. The lack of them is causing some disturbances in organism because the animals are not able to synthetize them. In experiments where diets were deficient lipids there appear there some symptoms of diseases which disappear after adding linoleic, linolenic or arachidonic acids. Linoleic acid possesses nearly six-times greater activity than linolenic acid - it comes out of its configuration /6, 12, 20/. There are much greater amount of unsaturated fatty acids in insect lipids than in mammalians /16, 24/. It shows directly that these compounds are very important for insects. The experiments on insects have demonstrated that there were great disturbances in Corcyra cephalonica St. and Ephestia development when the deficient unsaturated fatty acids diet was applied /caterpillars reached only chrysalis stage -/21, 16/. By adding simultaneously an alfa tocopherol a succesful activity on unsaturated fatty acids was observed. This was an effect of a protective action of alfa tocopherol as antioxidant of unsaturated compounds /16/. W i g g l e s w o r t h /24/ suggested that in insects the fundamental function of tocopherol is protective action against unsaturated fatty acids. Tocopherols participate in many metabolic processes in animals organism. All changes in tocopherols contents in food appear in biological tests as disturbances on genital system /26/. Therefore the most conveniant means for studying the activity of tocopherols on animals is to make record of data of multiplying capacity of the objects breeded with foods enriched or deficient by vitamin E. In investigations on the effect of alfa tocopherol on the fertility of Colorado beetle Z w o l i ń s k a - Ś n i a t a ł o w a /27/ stated that the alfa tocopherol factor has a positive influence on the increase in eggs laying by female beetles.

Having in view a great physiological importance of tocopherols and unsaturated fatty acids for the insects, we have begun to carry out the researches which will explain the role of these compounds in Colorado beetle development. The second problem deals with the finding out of an existing interdependence between the alfa tocopherol and unsaturated fatty acids in their effect on the insects.

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2. Experimental procedure

The biological experiments on larvae and beetles were carried out in insectarium in five different breeding combinations:

1/ We breed the larvae and the beetles with potato leaves enriched by adding alfa tocopherol,

2/ by adding unsaturated fatty acids,

3/ by adding the mixture of alfa tocopherol and unsaturated fatty acids,

4/ with the leaves sprayed only with the solvent - ether, and

5/ the control.

During the whole cycle of development of Colorado beetle we recorded data of the following observations: fertility, mortality, eating habits and other characteristics of their behaviour. On the larvae L_4 and on the beetles from five breeding combinations the following analyses have been made: finding of total lipids /linoleic, linolenic, arachidonic, pentaenoic/ and quality chromatographic determination of composition of fatty acids. The determination of total tocopherols in the hemolymph and in the whole body of the larvae L_4 /breeded only with leaves of potato Dar variety/ was made too.

Materials and methods

Since the 15th May we were collecting beetle eggs on our experimental fields and then we put them into the glass plates in insectarium at temperature 25°C. Hatched larvae were put into the special ring glasses /Fig.3/ with the net covers and breeded them according our method, in five breeding combinations. For breeding experiments we use the leaves of Dar potato variety planted only for this purpose. The dates of planting period were 21th April, 26th May, 17th June. During the vegetation period of potatoes some data of the observations were made. Potatoes were not sprayed or treated besides the usual agriculture works. To obtain repeatable results we always used for breeding the same kind of leaves: the whole top of plant included the fourth leaf. The leaves were enriched by means of spraying them with:

1/ acetate alfa tocopherol, average amount 3mg% - this corresponded to average contents of tocopherols in 4-th leaf in the blooming period /26/.

2/ For enrichment with unsaturated fatty acids we have used the extract from oleaginous plant named "Krokosz" which has a great number of unsaturated fatty acids /iodine number 229,37/. The doses applied were almost the same as the contents of unsaturated fatty acids in leaves /10/.

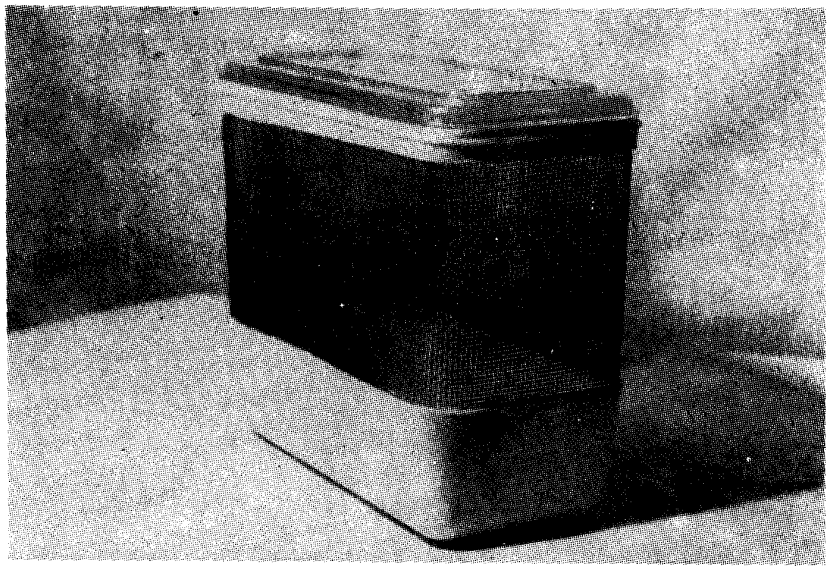


Fig. 1 - Great isolator

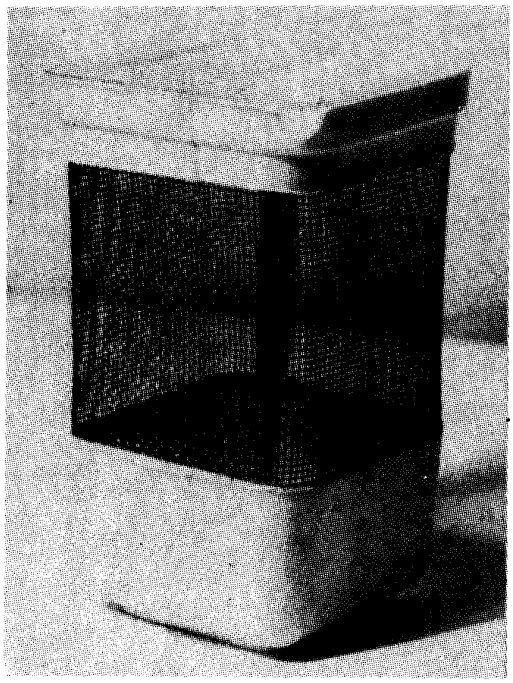


Fig. 2 - Small isolator

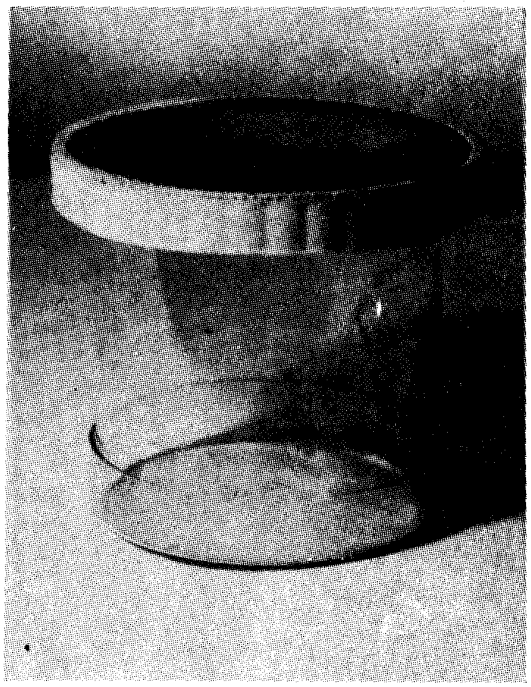


Fig. 3 - Glass ring

We begun our biological investigations the 13th of June on the larvae L_1 and 21-th of July with the beetles. For all breeding treatments period from the hatching time of larvae L_1 to the moment of appearance of the young beetles on the surface of sand lasted 39 days. After hatching the beetles were put into 4 great isolators /Fig.1/ - 10 female beetles and 5 male into each of them - and into 10 small isolators /Fig.2/ the couples the beetles. Together 30 beetles in each breeding combination. The net cages /named "isolators"/ were made of plastic and metalic net combinations. In isolators the layer of sand was 2,5 cm thick and the leaves were put on the surface in the little glasses. Into the great isolators we put the leaves from the top /included the fourth leaf - together the 20 g/ and into the small only 2 single leaves taken from 4-th leaf. The surface of leaves of small isolators was measured by planimeter instrument before putting them inside and on the next day after taking them out.

In breeding combination with unsaturated fatty acids after hatching the beetles, it was stated, that the number of male was greater than the number of female beetles. Therefore in this combination there were only great isolators. We have started with the experiments with young beetles 21-th of July and ended them about the 10-th of September. When all beetles went for their hibernation we have separated by means of wire sieve alive beetles from the dead and found the ratio between them. The beetles from all breeding treatments were taken for analyses.

A n a l i t i c a l m e t h o d s

Determination of total lipids.

The determination of total lipids was made by means of rest after extraction method. Ethyl ether dried and free from peroxides /18/ was used as a solvent. The time of extractions in Soxhlet apparatuses was 30 hours.

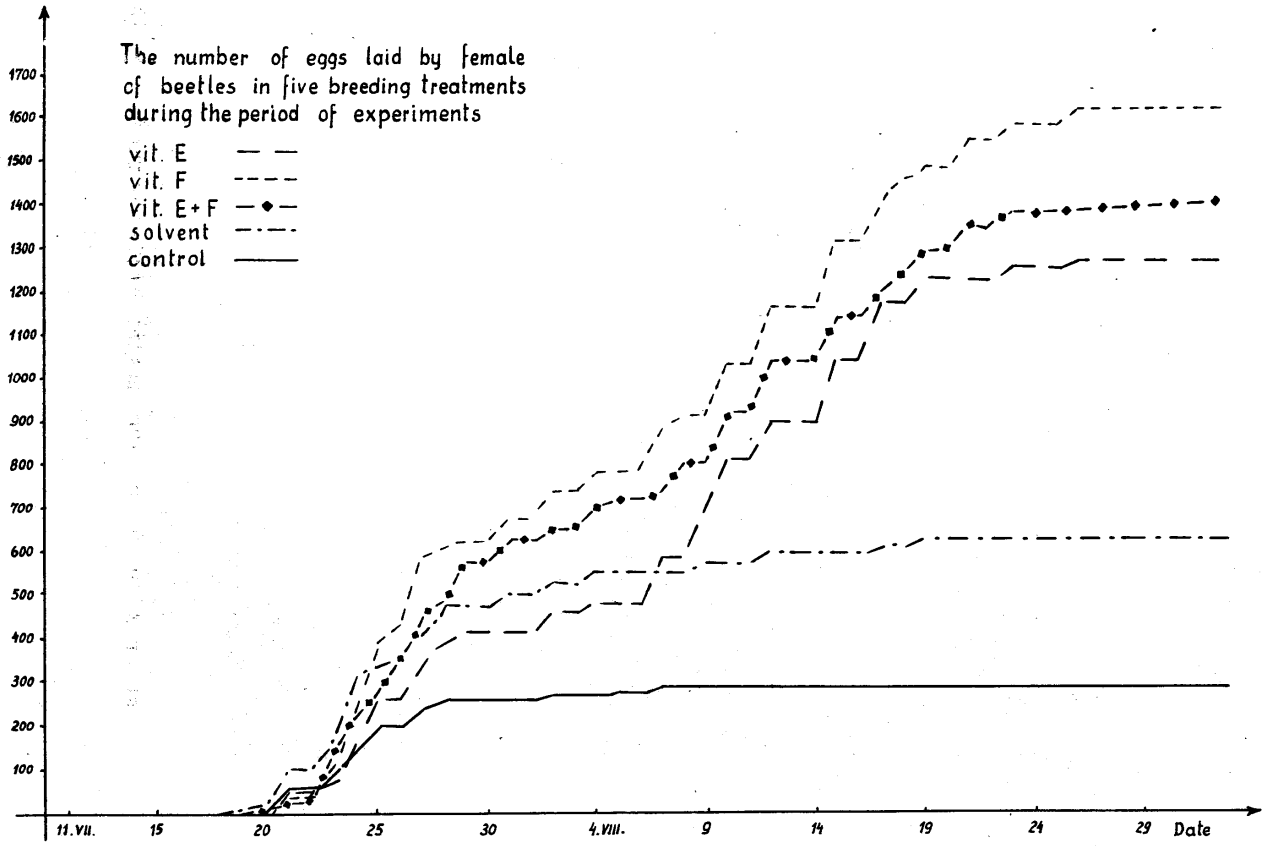
Determination of iodine number

For the determination of iodine number the bromo-piridine method of N i e m i e r k o /14/ was adapted. About 5 mg of lipids taken from the chloroform solution and 2 ml bromopiridine reagent were remained in darkness for 15 minutes. Next 1 ml of 10% KJ was added and total content was titrated by 0.05 N sodium thiosulphate in the presence of starch.

Spectrophotometric determination of unsaturated fatty acids

10 mg unsaturated fatty acids with 21% KOH /dissolved in ethylene glycol/ were isomerized by heated in 180°C during 15 min. Then the solution was cooling rapidely and

Figure IV



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methyl alcohol was added to 50 ml of volume. The mixture was kept in temperature of -2°C to the next day. Optical density was measured at 234 mμ, 315 mμ, 346 mμ. The content of linoleic, linolenic, arachidonic and pentaenoic acids was calculated from the respective formulas /8, 17/.

Quality chromatographic determination of fatty acids

The fatty acids were separated by paper chromatography. The method of reversed phases have been developed. As the mobile phase 90% acetic acid was used, and as a immobile carbohydrides b.p. 185-205°C. On the developed chromatograms the fatty acids are changed into the coloured cupric salts /11/.

Determination of total tocopherols

To determination of total tocopherols Emmer-Engel method in B l a i m /3, 4/ modification was used. The sample was saponified with 1N KOH in methyl alcohol by heating in 70°C in CO_2 stream through 15 min. Unsaponifiable materials were extracted by petroleum ether. Coloured, interfered substances in benzene solution were absorbed by rejowiecka soil on the standard column. Measurements were made on spectrophotometer SF 4 at 520 mμ.

3. R e s u l t s

The biological experiments

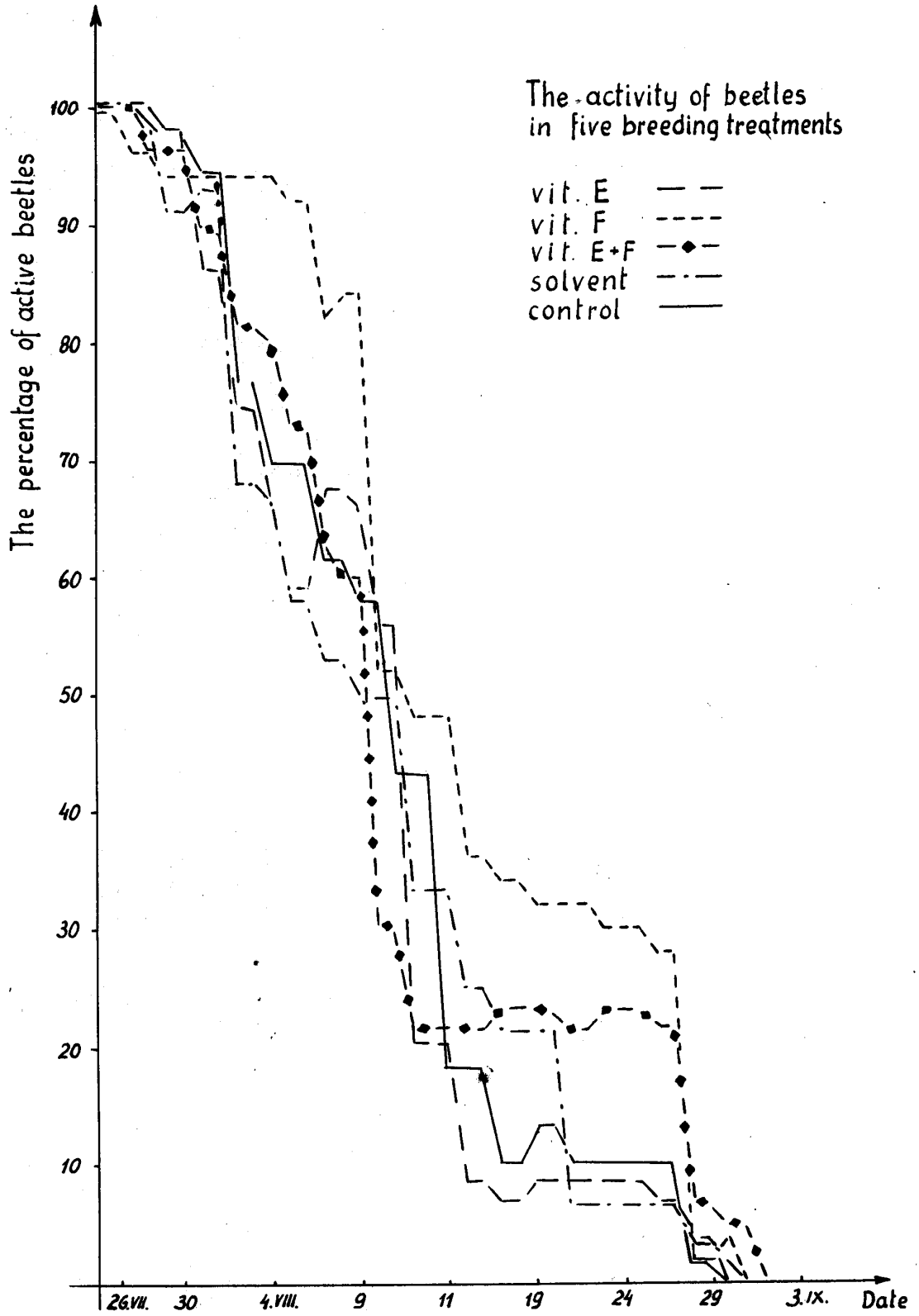
The number of eggs laid by the female beetles in five breeding treatments are presented in Fig.IV and Tabl.I. In the Fig.IV we can see the curves represented the number of eggs laid by the female beetles of five breeding treatments during the whole period of experiments. As it has been shown in breeding treatments enriched with vit. E and F, the period of intensive eggs laying was prolonged. In the control the maximum of eggs laid fall in the last days of July while in the vitamin treatments about 20-th of August.

Table I presents the number of eggs /mean for one great isolator/ laid by the female beetles in breeding treatments and the logarithms of means for statistical data.

T a b l e I

Treatment	means	logarithm of means
vit. E tocopherol	421,0	2,5713
vit. F unsaturated fatty acids	482,0	2,6432
mixture vit. E + vit. F	394,0	2,5734
solvent	187,7	2,2693
control	90,7	1,9272

Figure V



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Allowance for differences from the control /P=95%/ two sided A_2 = 0,4838. Analysis of variance made for logarithms of means shown, that there are significant differences between the breeding treatments and the control.

Activity of the beetles

Fig. V presents curves of activity of the beetles in five breeding treatments. As it can be see there are no significant differences in the activity of the beetles. 50% of all beetles went for hibernation about 10-th August and 100% about 30-th of August.

Mortality

In the period of biological experiments the mortality was among the beetles insignificant.

Eating habits

Table II presents the whole quantity of eaten leaves in mm^2 by all the beetles in the breeding treatments.

T a b l e II

Treatment+	Quantity of eaten leaves by the beetles in mm^2
vit. E tocopherol	195435
mixture vit.E + vit.F	218725
solvent	218800
control	228799

+ As it was explained above, there were no beetles in small isolators in combination with unsaturated fatty acids.

Analysis of variances has shown, that there are no significant differences between the breeding treatments. The variance ratio

$$F = 1,11 < 2,26 = F_{0,05}$$

Table III presents mean quantity of eaten leaves by the couple of beetles in mm^2.

statistical elaboration. Results presented in table IV allow however to admit that supplemented food for Colorado beetle with alfa tocopherol /vitamin E/, unsaturated fatty acids /vitamin F/ and the mixture of both, caused the increase in unsaturated fatty acids in the larvae lipid. To obtain the data about a content of tocopherols in hemolymph and in the whole body in larvae, the determination of total tocopherols was made. Results of analyses have shown that in the whole body of the larvae L-4 the content of tocopherols amounted in average to 1,84 mg% and in the hemolymph 3,70 mg%.

Table V presents the results of the determination of total lipids and unsaturated fatty acids in the beetles which are ready for the hibernation.

T a b l e V

Treatment	% of total lipids +	% of linoleic acid	% of linolenic acid	% of arachidonic acid	% of pentaenoic acids	S u m
vit.E	40,41	10,51	16,21	2,55	4,03	33,40
vit.F	41,97	10,78	15,23	2,25	3,12	31,38
vit.E+F	37,55	8,87	12,12	2,93	5,20	29,13
control	41,74	7,96	15,49	1,26	2,55	27,26
allowance for differences from the control P=95% two sided	3,03	1,51	2,37	0,60	0,40	3,02

+ The content of total lipids is calculated in dry weight of the beetles. The results are means from five determinations. Analysis of variance has shown, that there are significant differences between the beetles from breeding treatments and the control. The quality chromatographic analyses were made on the larvae L-4 and on the beetles before their hibernation. The results have shown, that the composition of fatty acids in the larvae and in the beetles was similar. In all combinations we have stated some quantity of saturated fatty acids /palmitic and stearic/, and unsaturated fatty acids /chiefly oleic, linoleic and linolenic/.

Table III

Treatment	Mean quantity of leaves eaten daily by couple of beetles in mm^2
vit. E tocopherol	857,17
vit.E + Vit.F mixture	825,38
solvent	931,06
control	879,99

Analysis of variance has shown that there are no significant differences between the means of breeding treatments. The variance ratio

$$F = 0,51 < 2,86 = F_{0,05}$$

Results of biochemical analyses

On 50 larvae from each breeding treatment the following analyses were made: finding of total lipids, spectrophotometric determination of linoleic, linolenic, archidonic and pentaenoic acids. Results are presented in Table IV.

Table IV

Treatment	% of total lipids+	% of linoleic acid	% of linolenic acid	% of arachidonic acids	% of pentaenoic acids	Sum of fatty acids
vit.E	2,33	26,30	28,27	8,64	8,55	71,96
vit.F	3,27	29,53	26,41	6,10	7,25	69,29
vit.E, vit.F	3,38	27,26	27,48	8,42	7,64	70,80
control	2,91	16,88	22,05	4,66	5,80	49,39

+ % of total lipids are expressed in fresh weight of the larvae.

Because of to small number of larvae I-4 it was impossible to repeat analyses. Therefore we didn't make the

pherol and unsaturated fatty acids there is the lower content of linolenic acid, but much higher of pentaenoic acid compared to the control. In general it was stated that the content of unsaturated fatty acids is much higher in the larvae L-4 than in the beetles. This shows that there is a great accumulation of these acids in the larvae stage and that they are used during the chrysalis period. The results of chromatographic analyses have shown that the composition of fatty acids in the beetles is similar to the larvae. The influence of these vitamins appeared most distinctly in imago stage. In all breeding treatments with vitamins the number of eggs laid by the female beetles was almost 4 times higher than in the control. In the similar way the intensive period of eggs laying was prolonged - and so the beetles from the control stopped laying eggs on the 5-th August, and in the breeding treatments with vitamins on the 30-th August. This is more peculiar because the activity period of the beetles of all these treatments did not show any differences among them. And the mean quantity of food eaten daily by the beetles also did not differ between the treatments. These above results show us exactly that the addition to the food both tocopherol and unsaturated fatty acids is influencing positively on the fertility of Colorado potato beetle. We have established that the addition of unsaturated fatty acids cause an increase in fertility of Colorado potato beetle as being use alone and in the mixture with alfa tocopherol. Addition of unsaturated fatty acids to the food of Colorado beetle caused no avitaminose of tocopherol. It looks more likely that there is no direct action of unsaturated fatty acids on fertility of Colorado potato beetle. These acids work by mean of increasing of food quality.

5. Conclusions

1. All data reached in the biological experiments and in the biochemical analyses indicate clearly that tocopherol /vitamin E/ and unsaturated fatty acids /vitamin F/ are very important factors for the Colorado potato beetle development. This appears first of all a/ in an increased number of eggs laid, and 2/ in an increase in unsaturated fatty acids of larvae and beetles lipids.

2. It has been established also that the addition of unsaturated fatty acids to the Colorado potato beetle food did not caused any avitaminose E.

6. Plan for future work

To come to the final conclusion about the influence of tocopherol /vitamin E/ and unsaturated fatty acids /vitamin F/ on Colorado potato beetle development and about the interdependence of these constituents, in the coming period we intend to do some biological breeding treatment experiments

4. D i s c u s s i o n

For our investigations of the influence of some vitamins on the physiology of Colorado potato beetle we used the materials supplied from Institute of Plant Protection fields: the eggs collected on the plantations of the potato Dar variety, and the leaves of the same potato variety. The potato named "Dar" have been used in the past years for the researches purposes. The aim of these investigations was chacking the influence of some compounds which are in potato leaves on Colorado beetle /25, 19, 26, 9/. The results of these researches have allowed to define the forming of some constituents in potato Dar variety leaves in the vegetation period. It was stated there, that the greatest increase of tocopherols - about - 3,5 mg% - in potato leaves takes place especially during the blooming season or sometimes before it. During the vegetation period of potatoes the content of total lipids in the potatoes leaves Dar variety increased from 4% at the beginning to the 7% in autumn. Among the fatty acids contained in potato Dar variety leaves the saturated fatty acids amounted to 24% and unsaturated 76% /9/. It is noteworthy that during the whole vegetation period the ratio between the saturated and unsaturated fatty acids changes fundamentally. All above mentioned data became a starting point for calculating the quantity of supplemented vitamins in our breeding treatments. In our experiments we carried out the investigations from the larvae L-4. It was interested to see if this enrichment obtained by adding vitamins had any influence on the development of larvae in all 4 stages. There are some data in literature about the acumulating of total lipids in the larvae L-4 before the metamorphose. W i g g l e s w o r t h /24/ has pointed out that the acumulation of fats in larvae L-4 decide of the health of the beetles. K r z y m a ń s k a /9/ suggested that there is the greatest accumulation of lipids in the insects before the metamorphosis and diapause. In our experiments we have stated, that there are no great differences in the content of total lipids in larvae L-4 amoung the breeding combinations which amount in average 3% in fresh weight. As the results of our observations have shown, the development of the larvae followed the same way in all five breeding treatments. We have found in the larvae L-4 the higher content of unsaturated fatty acids in all breeding treatments than in the control larvae was. The smallest difference was stated in the case of linolenic acid and much higher in linoleic and arachidonic acids. These data show us that the addition of vitamin E and vitamin F caused the increase in the content of unsaturated fatty acids. We came to the same conclusion where we are discussing the results of the beetles analyses. The spectrophotometric determination of linoleic, linolenic, arachidonic and pentaenoic acids have shown that the content of these acids is higher in the beetles from breeding treatments with unsaturated fatty acids and tocopherol than in the control. In the beetles from the breeding treatments with mixture of toco-

on the larvae and on the adult beetles as we did last year. Apart of analyses performed this year, the determination of tocopherols in hemolymph in the larvae from five breeding treatments will take place.

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